PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P101116WO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04258	International filing date *(day/month/year)* 03.10.2003	Priority date *(day/month/year)* 07.10.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/68		
Applicant LUDWIG INSTITUTE FOR CANCER RESEARCH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.04.2004	20.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer van der Kooij, M Telephone No. +31 70 340-4606

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04258

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-20 as originally filed

Claims, Numbers

1-43 received on 27.08.2004 with letter of 27.08.2004

Drawings, Sheets

1/18-18/18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 44-45

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-43
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-13,41-43
	No:	Claims	14-40
Industrial applicability (IA)	Yes:	Claims	1-43
	No:	Claims	-

2. Citations and explanations

   see separate sheet

Re Item I

The amendments of claims 14-40 has introduced subject-matter which extends beyond the content of the application as filed, contrary to Article 19(2) PCT. The amendments (bold) concerned is the following "a polypeptide encoded by a nucleic acid molecule which hybridises **under stringent hybridisation conditions** to the nucleic acid molecule in (I) **and is a polypeptide that induces the apoptotic function of p53**".
The stringent hybridisation condition for a nucleic acid encoding the polypeptide is only disclosed in relation to a preferred method of the invention and not in relation to the use of the polypeptide in a treatment or the use of an antagonist which interacts with a polypeptide in a treatment (page 3, lines 15-17).
Similarly, the fact that the polypeptide has to induce the apoptotic function of p53 is not disclosed in relation to the use of said polypeptide.
Therefore, subsequent report has been established as if said amendments had not been made.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V-1). Prior art documents.

Reference is made to the following documents:

D1: Z. Tan, et. al., *Brain Research*, 1 March 2002, Vol. 929, pages 129-138.
D2: WO-A-0212325
D3: Y. Samuels-Lev, et al., *Molecular Cell*, 2001, Vol. 8, pages 781-794.
D4: WO-A-9915657

The examination has been carried out assuming that the priority of the application is valid. However, the Applicant's attention is drawn to the fact that the documents which have been cited in the search report as "E" or "P" documents may become relevant in the national/regional examination phase.

V-2). Article 33(2) PCT.

Present claims 1-43 do meet the criteria of novelty set forth by Article 33(2) PCT. There is no prior art available disclosing a method for the detection of ASPP1 or ASPP2 in a nerve cell or a nerve progenitor cell or its use or an antagonist thereof for the manufacture of a medicament for use in the treatment of neurodegenerative diseases resulting from an abnormal expression of ASPP1 or ASPP2. Neither is a method for screening for agents known that modulate the activity of ASPP1 or ASPP2 by inducing the apoptotic function of p53 in nerve cells or nerve progenitor cells.

D1 discloses a method for the detection of Ref-1 in hippocampal CA1 pyramidal neurons (page 130, column 2, paragraph 3; Table 1 on page 132 and Figure 2). Ref-1 is a polypeptide that activates the p53 function leading to cell death in LPSE-induced neurodegeneration (see abstract and Introduction part and Figure 6). However, Ref-1 is not homologous to ASPP1 or ASPP2.

D2 discloses methods for screening for agents capable of modulating the activity of ASPP1 (SEQ.ID. NO: 13 is identical to the DNA sequence encoding ASPP1) or ASPP2 (SEQ.ID. NO: 14 is identical to the DNA sequence encoding ASPP2). Also, the polypeptides encoded by said DNA sequences or its nucleic acids are used in the manufacture of a medicament for modulating apoptosis (page 48, lines 23-24). However, there is no link given to neural development or neurodegeneration.

D3 discusses the importance of ASPP1 and ASPP2 in the activation of p53 responsive promoters in cancer cells (abstract). There is, however, no indication given of its role in neural development or neurodegeneration.

D4 discloses that p53BP2, a truncated version of ASPP1 and ASPP2, forms complexes with a variety of proteins. It is speculated that complexed p53BP2 may be involved in neurodegeneration. There is no suggestion on the role of the uncomplexed p53BP2 in neural development or neurodegeneration.

V-3). Article 33(3) PCT.

V-3.1). Present claims 1-13 and 41-43 do meet the requirements of Article 33(1) PCT, because the subject-matter of these claims involves an inventive step as set forth by Article 33(3) PCT.

D2 is considered to represent the most relevant state of the art and discloses the polypeptides identical to ASP1 and ASP2 (see Figure 1c and 1d, respectively; claims 1-5) capable of inducing the apoptotic function of p53, an assay for detecting the polypeptide in a sample (claims 42-46), an assay for detecting the presence of the

nucleic acid encoding the protein in a sample (claims 6-10, 36, 37 and 47-52) and a method to screen for agents which modulate the activity of the polypeptide (page 9, lines 16-page 10, line 12) suitable as anticancer agents.
The subject-matter of claims 1-43 differs in that the polypeptides appear to have their action in the neural development.
The problem to be solved by the present invention may therefore be regarded as the provision of a screening method for detection of said polypeptide and to identify agents that modulate the activity of said polypeptide in abnormal development of the nervous system.
The solution to this problem proposed in claims 1 and 41 of the present application is considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
The prior art lacks information on the involvement of ASPP1 and ASPP2 in neural development. Because there is no incentive for the role ASPP1 and ASPP2 play in neural development, the subject-matter of claims 1 and 41 and of its depending claims 2-13 and 42-43 of the present application is considered as involving an inventive step.
V-3.2). Present claims 14-40 do not meet the requirements of Article 33(1) PCT, because the subject-matter of these claims do not involve an inventive step as set forth by Article 33(3) PCT.
D2 is also considered to represent the most relevant state of the art for claims 14-40 and discloses the polypeptides identical to ASP1 and ASP2 (see Figure 1c and 1d, respectively; claims 1-5) capable of inducing the apoptotic function of p53.
A truncated version, p53BP2 falls however under the definition of "a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (I)" and has been implicated in complex with other proteins in disorders of neurodegeneration (**D4**: page 40, lines 3-20). Under normal circumstances, a protein often complexed with other proteins in a cell, so that it is not inventive to use the sole protein for the same purpose or for treating disorders of neurodegeneration. Therefore, the subject-matter of claim 14 -40 is regarded as obvious and does not involve an inventive step.

V-4). Article 33(4) PCT.
Claims relating to methods for detection or for identifying a potential therapeutic agent or its use in the manufacture of a medicament in the treatment of a disorder according to claims 1-43 are generally considered as industrial applicable since they can be made

or used in industry or agriculture. Therefore, claims 1-43 are considered to fulfill the requirements of Article 33(4) PCT.

V-5). Certain observations.

Method claims 15-17 are dependent on claim 14 which appears to be a use claim.

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CLAIMS

1 A method for the detection of a polypeptide in a cell or tissue sample which sample comprises a nerve cell or a nerve progenitor cell and wherein said polypeptide is a polypeptide which induces the apoptotic function of p53.

2. A method according to Claim 1 wherein said polypeptide is selected from the group consisting of:

a) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

b) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (a); or

c) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (a) and (b); said method comprising the steps of;

i) providing a sample comprising a nerve cell or a nerve cell progenitor cell;

ii) contacting said sample with an agent which binds said polypeptide;

iii) detecting the presence of said polypeptide in said cell sample.

3. A method according to Claim 2 wherein said polypeptide is encoded by a nucleic acid molecule which hybridises under stringent hybridisation conditions to the nucleic acid sequence as represented in Figure 1 or 2.

4. A method according to Claim 3 wherein said nucleic acid is represented by the nucleic acid sequence in Figure 1 or 2.

5. A method according to any of Claims 2-4 wherein said polypeptide is represented by the amino acid sequence in Figures 3 and 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

6. A method according to any of Claims 1-5 wherein said agent is an antibody which binds said polypeptide.

7. A method according to Claim 6 wherein said antibody is a polyclonal antibody.

8. A method according to Claim 6 wherein said antibody is a monoclonal antibody.

9. A method according to any of Claims 6-8 wherein said antibody is provided with means which enable the detection of the antibody bound to said polypeptide.

10. A method according to Claim 9 wherein said detection means is selected from the group consisting of: an enzyme; a isotope label or a fluorescent label.

11. A method according to any of Claims 1-5 wherein said method is the detection of a nucleic acid molecule which encodes said polypeptide.

12. A method according to Claim 11 wherein said agent is a nucleic acid molecule adapted to anneal to said nucleic acid molecule which encodes said polypeptide.

13. A method according to Claim 12 wherein said nucleic acid molecule is at least one oligonucleotide molecule.

14. A method according to Claim 13 wherein said nucleic acid molecule is a pair of oligonucleotide molecules adapted to bind said nucleic acid molecule which is to be detected.

15. A method according to Claim 14 wherein said method is a polymersase chain reaction method.

16. The use of a polypeptide selected from the group consisting of:

i) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

ii) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (i); or

iii) a polypeptide encoded by a nucleic acid molecule which is degenerate because of the genetic code to the nucleic acid molecule represented in (i) and (ii)..

for the manufacture of a medicament for use in the treatment of neurodegenerative diseases which result from abnormal expression of said polypeptide.

17. A method according to Claim 16 wherein said polypeptide is encoded encoded by a nucleic acid molecule.

18. A method according to Claim 16 or 17 wherein said polypeptide is represented by the amino acid sequence in Figures 3 and 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

19. A method according to Claim 17 wherein said nucleic acid molecule is part of a vector adapted for gene therapy.

20. The use of an antagonist which interacts with a polypeptide selected from the group consisting of:

i) a polypeptide encoded by a nucleic acid molecule as represented by the sequence in Figure 1 or 2;

ii) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (i); or

iii) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (i) and (ii).

for use in the manufacture of a medicament for use in the treatment of neurodegenerative diseases which result from abnormal expression of said polypeptide.

21. Use according to Claim 20 wherein said polypeptide is represented by the amino acid sequence in Figures 3 and 4 wherein said sequence has been modified by addition, deletion or substitution of at least one amino acid residue.

22. Use according to Claim 20 or 21 wherein said disease is selected from the group consisting of: Alzheimer's disease; Parkinson's disease; multiple sclerosis; or a retinopathy.

23. Use according to any of Claims 20-22 wherein said antagonist is an antibody or antibody part which binds said polypeptide.

24. Use according to Claim 23 wherein said antibody is a monoclonal antibody or binding part thereof.

25. Use according to Claim 23 or 24 wherein said fragment is a Fab fragment.

26. Use according to Claim 25 wherein said fragment is selected from the group consisting of: $F(ab')_2$, Fab, Fv and Fd fragments; and CDR3 regions.

27. Use according to any of Claims 24-26 wherein said antibody is a humanised.

28. Use according to any of Claims 24-26 wherein said antibody is a chimeric antibody.

29. Use according to Claim 20 wherein said antagonist is a nucleic acid molecule.

30. Use according to Claim 29 wherein said nucleic acid molecule is a transcription cassette comprising an nucleic acid molecule operatively linked to a promoter which promoter transcribes said nucleic acid molecule to produce an antisense nucleic acid molecule, said sequence selected from the group consisting of:

i) a nucleic acid sequence, or part thereof, as represented in Figure 1 or 2;

ii) a nucleic acid sequence which hybridises to the sense sequence presented in Figure 1 or 2 and which encodes a polypeptide with anti-apoptotic activity.

31 Use according to Claim 30 wherein said cassette is part of a vector.

32. Use according to Claim 29 wherein said nucleic acid molecule comprises a transcription cassette wherein said a nucleic acid molecule, or part thereof, selected from the group consisting of:

i) a nucleic acid molecule represented by the nucleic acid sequence in Figure 1 or 2;

ii) a nucleic acid molecule which hybridises to the sequences in (i) above and which encodes a polypeptide with anti-apoptotic activity; or

iii) a nucleic acid molecule which is degenerate as a consequence of the genetic code to the sequences defined in (i) and/or (ii) above; wherein said cassette is adapted such that both sense and antisense nucleic acid molecules are transcribed from said cassette.

33. Use according to Claim 32 wherein said cassette is provided with at least two promoters adapted to transcribe both sense and antisense strands of said nucleic acid molecule.

34. Use according to Claim 32 wherein said cassette comprises a nucleic acid molecule wherein said molecule comprises a first part linked to a second part wherein said first and second parts are complementary over at least part of their sequence and further wherein transcription of said nucleic acid molecule produces an RNA molecule which forms a double stranded region by complementary base pairing of said first and second parts.

35. Use according to Claim 34 wherein said first and second parts are linked by at least one nucleotide base.

36. Use according to Claim 35 wherein said first and second parts are linked by 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 nucleotide bases.

37. Use according to any of Claims 32-36 wherein the length of said RNAi molecule is between 100bp-1000bp.

38. Use according to Claim 37 wherein the length of said RNAi molecule is selected from at least 100bp; 200bp; 300bp; 400bp; 500bp; 600bp; 700bp; 800bp; 900bp; or 1000bp.

39. Use according to any of Claims 32-36 wherein said RNAi is at least 1000bp in length.

40 Use according to any of Claims 32-36 wherein said RNAi molecule is between 15bp and 25bp in length.

41. Use according to Claim 40 wherein said RNAi molecule is 21bp in length.

42. Use according to any of Claims 32-41 wherein said cassette is part of a vector.

43. A method to screen for agents which modulate the activity of a polypeptide which induces the apoptotic function of p53 comprising the steps of:

i) providing a cell sample comprising a nerve cell or nerve progenitor cell;

ii) contacting said sample with an agent to be tested; and

iii) monitoring effect of said agent on the presence and/or activity of said polypeptide.

44. A method according to Claim 43 wherein said polypeptide is selected from the group consisting of:

a) a polypeptide encoded by a nucleic acid molecule as represented by the sequence shown in Figure 1 or 2;

b) a polypeptide encoded by a nucleic acid molecule which hybridises to the nucleic acid molecule in (a); or

c) a polypeptide encoded by a nucleic acid molecule which is degenerate to the nucleic acid molecule represented in (a) and (b).

44. A method according to Claim 43 or 44 wherein said agent is an antagonist of said polypeptide.

45. A method according to Claim 43 or 44 wherein said agent is an agonist of said polypeptide.